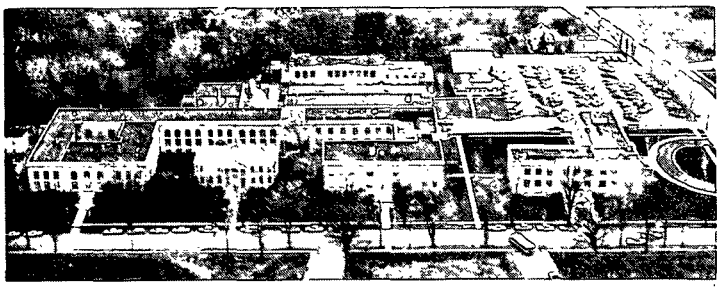

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MYCELIAL PAPER: A POTENTIAL RESOURCE RECOVERY PROCESS?

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INTRODUCTION

The idea that it might be possible to make sheets from fungal mycelia was investigated at The Institute of Paper Chemistry over 20 years ago. Data obtained at that time were sufficient to result in a patent, but the process remained essentially a novelty. Against a background of forecasts for fiber shortages near the coming turn of the century and of waste disposal problems already evident in this decade, mycelial paper now merits further consideration in terms of possible practical applications in the not-so-distant future. The investigation reported here was conducted to see if more than novelty interest in mycelial paper was justifiable and whether production of the mycelia might be bridged to waste disposal, perhaps contributing to the solution of two problems simultaneously.

The results of this research indicate that mycelia might at least be looked upon as a fiber extender in low level mixtures with wood fibers. Since there were observed differences in the behavior of mycelia from different organisms, further screening in this regard might be worthwhile. There are both chemical and physical aspects of the formation and properties of mycelial and mycelia-wood fiber sheets that would demand further attention if one were to consider any commercial applications. Observations presented here hopefully provide a foundation for more specific endeavors in the future.

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MYCELIAL PAPER: A POTENTIAL RESOURCE RECOVERY PROCESS?

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Summary

Eleven species of fungi representative of a broad range of cell wall compositions were evaluated with respect to their papermaking potential as additives to wood pulp furnishes. Some of these species were also examined for their ability to grow on a spent liquor from the pulp and paper industry. Handsheets with various levels of incorporated mycelia exhibited a wide range of species-dependent properties. Behavior of the mycelia in the sheets can be modified to a degree by physical and chemical treatments. The overall results suggest that small amounts (5-10% of the sheet constituents) of mycelia, grown inexpensively on waste effluents, might be incorporated into wood fiber paper without serious deleterious effects on paper strength properties. In some cases improved paper is obtained, and larger quantities of mycelia might be used to impart specific properties to the product.

INTRODUCTION

Several years ago a process for making sheets from fungal mycelia was developed and patented by The Institute of Paper Chemistry (1). In recent years we have reexamined this process from the point of view that the disposal of waste effluents with high biochemical oxygen demand might be coupled to the provision of fibrous materials useable in papermaking. This communication presents some of our observations which serve to illustrate what might be expected from such an approach with fungal mycelia as the product. The research of Bambacht (2) on the use of Achlya bisexualis as a beater additive to improve the strength properties of wood pulp is a rare, somewhat parallel investigation.

Some of the impetus to advance further probes into this area came from a growing literature on the cell wall composition of fungi, including a classification on this basis (3,4). That knowledge of the constituents of fungal cell walls continues to build is obvious from the review of Sturgeon (5) and recent detailed reports on specific organisms, e.g., Pythium debaryanum (6). The cell wall composition classes of Bartnicki-Garcia (3) originally formed the basis for our selection of fungi to be screened as representatives of a wide chemical spectrum of mycelia. However, it should be noted that this is not meant to imply that mycelial composition is always the governing factor determining sheet properties; in fact, our experience has been that physical factors may often dominate the picture, particularly via sheet formation.

MATERIALS AND METHODS

Fungi

Phytophthora cinnamomi (ATCC No. 11928), Phytophthora parasitica (ATCC No. 13614), Saprolegnia ferax (ATCC No. 10396), Mucor rouxii (ATCC No. 4855), and Pythium debaryanum (ATCC No. 9998) were purchased from the American Type Culture Collection. The Fusarium sp. originated in our own laboratories as a contaminant in cultures of Mucor remaining from the research on which the patent was based (1). The Mucor organism of the patent description is no longer extant among our cultures. In addition to the foregoing organisms which were used in gathering the data presented here, the following were also purchased and studied: Daedalea unicolor (ATCC No. 9405), Polyporus anceps (ATCC No. 13242), Schizophyllum commune (ATCC No. 9418, also known as Flammulina velutipes), and Trametes suaveolens (ATCC No. 9417). A culture of Rhizidiomyces sp. was obtained gratis from Dr. Melvin S. Fuller of the University of Georgia. While not appearing in this report, some data are also available from these latter five organisms and may be obtained by writing to the authors.

Media

With the exception of S. ferax, the organisms were grown on defined asparagine-glucose medium (4) unless ability to grow on waste effluent was under investigation. The glucose (40% w/v in distilled water) was autoclaved separately and then added to the rest of the medium which previously had been adjusted to pH 6.0 with KOH and autoclaved for 20 min at 121°C. For the culture of S. ferax the above medium was prepared containing 0.1% w/v yeast extract.

Culture

With the exception of some Fusarium which was grown in a minifermentor, all fungi were grown in 3-liter Erlenmeyer flasks containing 400 ml of medium. Growth periods ranged from 5-19 days depending upon the growth rate of the organism. The Erlenmeyers were inoculated, placed on a reciprocating shaker (50 cycles/min), and incubated at 29°C in the dark. Inocula were 1 or 2 ml of mycelial suspension from small suspension cultures maintained for this purpose; mycelia were dispersed by shaking with glass beads prior to withdrawing the inoculum. Growth in the minifermentor (Mini-Ferm Model M-1000, Fermentation Design, Inc., Allentown, PA) entailed greater agitation and aeration with a sparger.

When the growth period was complete, the shake flasks were transferred to a steam oven and steamed for 1 hour. Steamed mycelia were cooled, filtered with suction through qualitative filter paper on a Buchner funnel (some organisms cause problems here), and resuspended in 300 ml distilled water for washing. This slurry was beaten for 30 sec in a Waring Blendor or, preferably, for 200 counts in the British disintegrator. Following filtration, the washing procedure was repeated once more. Finally, the mycelia were filtered, washed with distilled water on the filter, removed, and lyophilized from a distilled water slurry. Freeze-dried weights were obtained prior to handsheet formation.

Handsheet Preparation and Evaluation

Unbleached aspen kraft pulp was beaten 15 min in a Valley beater under TAPPI Standard conditions. Freeze-dried mycelia were slurried and mixed with the aspen pulp in various proportions (usually 0, 5, 10, 20, 25, and 50% on an oven-dry fiber basis). The mixtures were given 600 counts in the British disintegrator before making 1.2 g handsheets according to TAPPI Standard T 205 m-58. Freeness and drainage times on the handsheet mold were recorded. Handsheets were examined visually and evaluated for basis weight, thickness, density, moisture, burst, tensile, stretch, and tear by TAPPI Standard Method T 220 m-60. Bendtsen porosity was determined in ml/min for a 10 cm^2 area at air pressure for 150 mm of water.

Chemical Analyses

Neutral sulfite semichemical (NSSC) spent liquor was obtained from Green Bay Packaging Inc., Green Bay, Wisconsin. Methods used in analysis of this material are given with the tabulated data. Culture filtrates obtained following growth of organisms on the NSSC spent liquor were analyzed for acetate, carbohydrates, and lignin-related substances. Acetic acid analysis was conducted by direct gas chromatography of culture filtrates. Twenty microliters of culture filtrate acidified with phosphoric acid to pH 2 were injected onto a 6 ft x 1/8 inch stainless steel column packed with Porapak Q and fitted with a removable 6-inch precolumn of the same type. A glass insert was used in the injector port to accumulate nonvolatiles and replaced periodically. Analysis was conducted at a column temperature of 170°C, injector at 200°C, thermal conductivity detector at 285°C, and a helium flow rate of 25 cc/min. Quantitation was by measurement of the acetic acid peak areas with a planimeter with reference to an acetic acid standard. Retention time was 9.3 min.

The disappearance of phenolic compounds was followed simply by examination of the UV spectra of culture filtrates. The UV spectrum of the spent NSSC liquor had a broad peak at 270 nm. While more than lignin may easily be contributing to that absorbance, this monitoring device did provide a convenient estimate of utilization of UV-absorbing compounds by the organisms; the procedure could be refined in cases of special interest. Sugar analysis was conducted by an indole colorimetric procedure for total carbohydrate (7). A breakdown of the carbohydrate analysis of the spent liquor showed the following percentages of the various sugars: rhamnose, 0.106%; arabinose, 0.111%; xylose, 0.564%; mannose, 0.029%; galactose, 0.180%; and glucose, 0.122%. The indole colorimetric peaks for equivalent concentrations of each of these sugars and for the mixture encountered in the NSSC spent liquor are given in Fig. 1. Disappearance of carbohydrate was therefore estimated from loss of absorbance at 470 nm.

[Fig. 1 here]

The scanning electron micrograph was obtained with a JEOLCO JSM-U3.

RESULTS

The freeness and the handsheet mold drainage time data for the several organisms at various pulp-mycelia mixtures are presented in Table I. Table II lists several properties of the resulting sheets. The results of burst, tensile, stretch, tear, and porosity tests are graphed in Fig. 2-6.

[Table I-II, Fig. 2-6 here]

Some of the operational parameters that accompanied the preparation of mycelial paper were investigated further. For example, the effect of employing a stronger control pulp in the preparation of mycelia-wood pulp mixtures for handsheet formation was investigated using P. debaryanum mycelia combined with

aspen pulp versus spruce kraft (Oxford) pulp. The handsheet evaluation data for aspen-mycelia and spruce-mycelia mixtures are compared in Table III. A study was also made of the effect of "beating" time of the mycelia-wood pulp mixtures upon their subsequent behavior. An arbitrary 600 counts in the British disintegrator was adopted for routine preparations as noted previously. This study of "beating" time was conducted with a mixture of 20% S. ferax mycelia and 80% aspen kraft pulp versus a 100% aspen control over a range of 50 to 3000 counts in the British disintegrator. The freeness and drainage results are given in Table IV; the handsheet evaluation data appear in Table V. S. ferax mycelia were also used to study the effect of lyophilization of mycelia on subsequent behavior of mycelia-wood pulp mixtures. The freeness and drainage data for freeze-dried versus never-dried mycelia are presented in Table VI; the handsheet evaluation data appear in Table VII.

[Tables III-VII here]

Some effort was expended to determine the feasibility of growing these organisms on waste effluent. The NSSC spent liquor used had a pH of 5.9 as received. Dilutions of this liquor were used both on the as-received basis and following centrifugation (10,000 x g for 15 min). In the latter case the resulting supernatant was also subsequently filtered through Whatman No. 1 paper since decantation was not a clean operation. Chemical analysis of this filtered supernatant appears in Table VIII. A growth curve and accompanying pH changes for Fusarium on NSSC spent liquor diluted 1:3 with distilled water (i.e., 25% NSSC) are shown in Fig. 7. Analyses of aliquots of culture filtrate for utilization of carbon sources resulted in the data of Fig. 8 which correspond to the events in Fig. 7.

[Table VIII, Fig. 7-8 here]

Scanning electron micrographs illustrating sheet formation in mycelial paper appear in Fig. 9.

[Fig. 9 here]

DISCUSSION

For the purpose of this investigation, no serious effort was made to optimize the growth of the organisms on defined media. The culture systems used did provide sufficient mycelia in a reasonable time to allow preparation of hand-sheets for examination. Steaming of the fungi or some alternative for killing mycelia (and spores, if a problem) would be essential if the organisms were serious pathogens and probably desirable in any case. In fact, additional studies indicate that further treatment with alkali to remove protein may improve the sheet-forming properties of the mycelia.

The freeness and drainage data for mycelial papers generally show that the addition of mycelia to wood pulp is much like a substitute for beating the wood pulp. Freeness drops and drainage resistance increases (Table I). The extreme case of this is P. debaryanum while M. rouxii and P. cinnamomi show much less effect. Beating the mycelia-wood mixture itself (Table IV) did not produce much additional effect. Freeze-drying the mycelia appeared to shorten the drainage time with high levels of mycelia present in the case of S. ferax (Table VI). The paper evaluation results (Table II and Fig. 2-6) show uniformity of all organisms with regard to a general loss of tearing strength as the percentage of mycelia is increased. Where bursting strength increases (F. sp. and S. ferax), there seems to be a corresponding definite increase in stretch. On the other hand, where burst values are maintained or decline, the stretch values fluctuate and definite trends are not established. S. ferax is the only organism causing an increase in tensile strength; Fusarium shows little change while mycelia of all other organisms lead to decreases in tensile. Porosity decreased rather

rapidly for all mycelial additions except for P. cinnamomi and M. rouxii both of which were poor performers in the strength tests. Basis weight values were quite uniform, and thickness, density, and moisture values appear to be consistent with other properties of the sheets. For example, where the porosity did not exhibit a rapid decrease (P. cinnamomi and M. rouxii), the sheet density declined or held relatively constant; density increased in the four other cases.

Other interesting sheet properties were observed, some of which are not evident from the data presented. The scanning electron micrographs in Fig. 9 do indicate the filamentous nature of the mycelia and an apparently bonded network with the larger aspen fibers. The small diameter of the mycelial filaments relative to the wood fibers undoubtedly plays a role in sheet formation and resultant sheet properties. It is evident from the results in Table III that P. debaryanum mycelia incorporated into handsheets impart essentially identical changes in strength characteristics of the sheets regardless of the strength of the control pulp with which they are mixed. Apparently, the mycelial strands tended to bond among themselves to about the same degree independent of the presence of the aspen or spruce fibers. Together with the electron micrographs these data suggest a predominance of intramycelial bonding in the sheets although some mycelia to fiber bonding does occur. Due to the substantial disparity in size it would seem that some pretreatment of the wood pulp to generate greater fibrillation might lead to the formation of more mycelia to fiber bonds and improve the sheet. Pretreatment of the mycelia has already been noted. At high levels of mycelia incorporation most handsheets take on a glassine nature. It is possible to make 100% sheets from some organisms but one can expect a certain degree of technical difficulty. Such sheets are usually glassine and very brittle. Sheet properties like opacity, smoothness, and brightness were not tested in this investigation but might be of interest in some cases.

The results in Tables V and VII were obtained with S. ferax which was perhaps the most promising organism investigated. Neither modest "beating" of the mycelial-wood pulp mixtures nor freeze-drying the mycelia before use appeared to be very significant variables.

It should also be mentioned that some mycelial papers have an offensive odor which tends to dissipate upon storage. Such a problem could no doubt be countered in several ways but protein removal via alkali treatment, noted above, should help considerably.

Growth of these organisms on spent liquor was given some attention because it appeared that, at least at the present time, mycelia could only be used as a fiber supplement if the economics were very favorable. Although S. ferax was a promising organism in the sheet property studies, it will be recalled that its growth, even on defined media, required supplementation with yeast extract. Likewise it would not grow on simple dilutions of the NSSC spent liquor. On the other hand some of the other organisms would grow on rather concentrated spent liquor without supplementation, and, on this basis, Fusarium and Mucor were singled out for additional study. It should be pointed out that, although clarified liquor (i.e., filtered supernatant as analyzed in Table VIII) was used in most of these investigations, it was usually not important in terms of growth but only as it facilitated some of the analyses. The growth curves and pH changes observed for Fusarium on 25% NSSC spent liquor without supplementation (Fig. 7) are typical responses that might be observed on defined media. The results in Fig. 8 indicate that the pH changes observed in Fig. 7 are due to the rapid catabolism of acetate coupled with slower degradation of the other substrates. Obviously, the growth of any organism on a waste effluent for this purpose would have to be examined specifically for each individual case. We found spent NSSC liquor to be inhibitory to different organisms at different dilution levels; usually at least a 1:1 dilution with water was required.

Supplementation of the spent liquor with a nitrogen and phosphorus source such as ammonium hydrogen phosphate was usually beneficial.

The organisms included in this communication fall into three general classes in terms of cell wall composition. The two Phytophthoras, Saprolegnia, and Pythium are all claimed to contain at least some cellulose in their walls, perhaps as high as 30-40% (3). Mucor belongs to a noncellulosic group where the polymers predominating are chitosan and chitin. Fusarium is a member of the higher fungi classed in the chitin-glucan group; most of this glucan appears to be other than β-1,4 linked although there is an unconfirmed report of cellulose in Fusarium (3). Organisms within the same class often performed quite differently in some of our testing. This may reflect the relative percentages of the various cell wall components in these cases, but it seems certain that in some cases it was a matter of sheet formation. For example, at high levels of Phytophthora incorporation sheet formation was visibly uneven with blotches of material scattered throughout the sheet. This factor would at least contribute to data scatter if not to overall performance in the paper evaluation tests.

Inspection of the data presented will no doubt reveal a number of shortcomings of mycelial paper. Nevertheless, such judgments would rest heavily on proposed uses for such a product. It appears that mycelia at low levels might be added to ordinary papers without serious consequences. Mycelia from an organism like Saprolegnia could be used to improve many properties of the paper. It is expected that eventually it may become profitable to explore the potential of this approach more thoroughly. Biomass production in this case might be shunted to help alleviate fiber shortages and pollution problems in addition to other looming problems with energy and food in which cultured fungi may also have a role to play (e.g., 8).

Acknowledgment

We gratefully acknowlege the contributions to this effort made by several other staff members of The Institute of Paper Chemistry during the preparation and evaluation of mycelial paper. The electron micrographs were courtesy of the laboratory of Dr. R. A. Parham.

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TABLE I

FREENESS AND DRAINAGE FOR VARIOUS PULP-MYCELIA MIXTURES

Percentage Mycelia	Freeness, C.s., ml						Drainage Time, sec					
	P.p.[a]	P.c.	F. sp.	M.r.	S.f.	P.d.	P.p.	P.c.	F. sp.	M.r.	S.f.	P.d.
0 (control)	500	500	490	490	490	490	5.7	5.5	5.6	5.6	5.3	5.3
5	400	480	405	445	415	425	7.0	5.8	7.0	6.3	6.5	6.7
10	300	450	330	435	365	375	8.7	6.2	8.7	6.8	8.3	8.3
20	215	410	220	380	275	240	13.0	6.8	12.7	7.2	12.2	14.2
25	195	380	195	360	240	195	15.2	7.4	17.0	7.6	16.1	18.5
50	120	275	90	310	140	75	26.6	9.1	27.0	N.D.[b]	34.0	53.0

[a] Organism abbreviations.

[b] N.D. = not determined.
C.s. = Canadian standard.

TABLE II

MYCELIAL PAPER EVALUATION DATA

Organism	Mycelia, %	Basis Weight, g/m^2, o.d.	Thickness, μm	Density, g/cc	Moisture, %	Burst Factor	Bendtsen Porosity, ml/min	Tensile, km	Stretch, %	Tear Factor
P. parasitica	0	60.7	85.6	0.709	7.6	49.7	221	9.37	1.6	79.7
	5	61.6	86.1	0.715	7.7	48.2	169	9.43	1.9	75.3
	10	59.7	82.3	0.725	8.0	48.5	120	8.93	1.8	65.7
	20	59.9	81.5	0.735	8.3	45.1	64	8.22	1.8	59.4
	25	61.6	80.9	0.761	8.3	46.6	41	7.14	1.5	55.2
	50	61.4	77.7	0.790	8.8	37.2	<12	6.37	1.4	43.6
P. cinnamomi	0	61.3	86.7	0.707	7.9	49.1	218	9.63	1.7	80.3
	5	61.2	88.5	0.692	8.0	46.6	171	9.29	1.8	74.5
	10	60.5	89.7	0.674	8.2	43.5	157	8.44	1.6	70.7
	20	59.7	90.5	0.660	8.5	37.4	161	7.57	1.5	62.3
	25	60.7	92.7	0.655	8.6	36.5	137	7.23	1.6	61.3
	50	60.8	97.1	0.626	9.7	26.5	108	5.20	1.6	42.1
Fusarium sp.	0	61.1	86.3	0.708	7.7	49.9	183	9.87	1.8	78.6
	5	62.0	85.8	0.723	7.8	50.9	133	9.90	1.8	72.9
	10	60.1	80.2	0.749	8.0	55.1	76	9.97	2.0	66.6
	20	60.6	80.0	0.758	8.3	55.0	42	8.75	1.8	62.0
	25	60.8	79.6	0.764	8.4	57.1	32	9.36	2.1	52.6
	50	62.2	80.3	0.775	9.2	45.2	<12	7.11	1.6	34.1
M. rouxii	0	61.1	86.3	0.708	7.7	49.9	183	9.87	1.8	78.6
	5	60.4	84.4	0.716	8.0	46.6	168	9.02	1.6	75.5
	10	60.5	84.2	0.719	8.2	43.6	160	8.74	1.7	72.7
	20	61.3	86.6	0.708	8.7	38.3	160	7.87	1.6	62.6
	25	60.7	84.3	0.720	9.0	36.1	155	7.24	1.5	60.0
	50	59.9	84.4	0.710	10.1	27.0	193	5.32	1.3	40.1
S. ferax	0	60.4	84.5	0.715	8.1	48.2	331	8.87	2.1	82.1
	5	61.0	84.0	0.726	8.3	48.3	211	9.02	2.3	80.0
	10	61.9	83.0	0.746	8.3	53.5	108	9.19	2.3	72.4
	20	60.2	78.1	0.771	8.9	55.6	31	9.36	2.4	63.1
	25	60.6	77.3	0.784	9.0	51.4	20	9.34	2.3	58.1
	50	60.0	70.3	0.853	10.0	40.3	<5	8.36	1.8	37.3
P. debaryanum	0	60.4	84.5	0.715	8.1	48.2	331	8.87	2.1	82.1
	5	60.2	82.8	0.727	8.2	43.2	231	8.75	2.2	76.4
	10	60.7	81.6	0.744	8.3	43.1	134	8.30	2.1	71.2
	20	60.1	75.5	0.796	8.6	46.8	39	7.91	2.0	59.2
	25	60.0	73.5	0.816	8.7	48.2	20	7.87	2.2	54.7
	50	60.7	68.7	0.884	9.3	36.7	<5	5.49	1.5	34.9

TABLE III

EFFECT OF CONTROL PULP IN MYCELIA-PULP MIXTURES

P. debaryanum Mycelia, %	Control Pulp	Basis Weight, g/m^2, o.d.	Thickness, μm	Density, g/cc	Moisture, %	Burst Factor	Bendtsen Porosity, ml/min	Tensile, km	Stretch, %	Tear Factor
0	Aspen	61.0	83.2	0.733	7.9	48.3	285	8.82	2.0	80.7
5		61.4	84.9	0.723	8.1	46.8	236	7.69	2.0	71.7
20		60.5	83.0	0.729	8.4	41.9	77	7.10	2.0	62.8
50		62.5	82.2	0.760	9.2	29.4	<12	4.71	1.3	36.5
0	Spruce	60.2	89.8	0.670	8.0	65.7	243	8.99	2.9	108
5		60.5	90.7	0.667	8.2	65.0	101	8.41	3.2	106
10		59.8	88.4	0.676	8.3	63.9	88	8.07	3.1	96.3
20		60.1	86.2	0.697	8.4	56.7	31	6.92	2.8	83.2
25		59.6	84.8	0.703	8.6	50.2	17	6.53	2.7	73.8
50		60.3	83.6	0.721	9.2	27.4	<12	3.92	1.1	N.D.[a]

[a] N.D. = not determined.

TABLE IV

FREENESS AND DRAINAGE OF A MYCELIA-PULP MIXTURE *VERSUS* BEATING TIME

S. ferax Mycelia, %	British Disintegrator, counts	Freeness, C.s., ml	Drainage Time, sec
0	50	505	5.2
	600	500	5.4
	3000	495	5.5
20	50	290	11.4
	300	280	11.9
	600	275	12.0
	1200	250	12.7
	3000	235	15.5

TABLE V

HANDSHEET EVALUATION DATA OF A MYCELIA-PULP MIXTURE *VERSUS* BEATING TIME

S. ferax Mycelia, %	British Disintegrator, counts	Basis Weight, g/m^2, o.d.	Thickness, μm	Density, g/cc	Moisture, %	Burst Factor	Bendtsen Porosity, ml/min	Tensile, km	Stretch, %	Tear Factor
0	50	59.6	84.9	0.702	8.1	44.1	369	8.79	1.9	88.6
	600	61.3	86.6	0.708	8.1	48.6	277	8.89	2.1	90.3
	3000	59.5	83.4	0.713	8.0	49.4	242	9.34	2.2	87.7
20	50	60.5	89.9	0.673	8.9	46.0	39	8.34	1.9	66.1
	300	61.1	89.4	0.683	8.8	47.1	28	8.60	2.0	66.0
	600	61.3	89.4	0.686	8.9	49.2	24	8.98	2.1	65.8
	1200	59.4	86.4	0.688	8.9	46.9	25	9.37	2.1	64.6
	3000	61.5	88.4	0.696	9.0	51.2	19	8.35	2.1	62.4

TABLE VI

FREENESS AND DRAINAGE FOR FREEZE-DRIED VERSUS NEVER-DRIED S. FERAX

Mycelia, %	Aspen, %	Freeness, C.s., ml	Drainage Time, sec
Freeze-dried			
0	100	490	5.3
5	95	415	6.5
10	90	365	8.3
20	80	275	12.2
25	75	240	16.1
50	50	140	34.0
Never-dried			
0	100	505	5.5
5	95	445	6.3
10	90	370	8.2
20	80	245	13.9
25	75	200	18.9
50	50	90	45.0

TABLE VII

HANDSHEET EVALUATIONS OF MYCELIAL PAPER MADE FROM FREEZE-DRIED VERSUS NEVER-DRIED S. FERAX

Mycelia, %	Aspen, %	Basis Weight, g/m^2, o.d.	Thickness, µm	Density, g/cc	Moisture, %	Burst Factor	Bendtsen Porosity, ml/min	Tensile, km	Stretch, %	Tear Factor
Freeze-dried										
0	100	60.4	84.5	0.715	8.1	48.2	331	8.87	2.1	82.1
5	95	61.0	84.0	0.726	8.3	48.3	211	9.02	2.3	80.0
10	90	61.9	83.0	0.746	8.3	53.5	108	9.19	2.3	72.4
20	80	60.2	78.1	0.771	8.9	55.6	31	9.36	2.4	63.1
25	75	60.6	77.3	0.784	9.0	51.4	20	9.34	2.3	58.1
50	50	60.0	70.3	0.853	10.0	40.3	<5	8.36	1.8	37.3
Never-dried										
0	100	59.8	82.3	0.727	7.5	51.7	319	8.76	2.1	85.6
5	95	60.5	81.0	0.747	7.6	54.0	111	9.49	2.4	78.7
10	90	61.1	80.8	0.756	7.8	54.2	46	9.46	2.4	70.0
20	80	60.7	77.9	0.779	8.2	57.0	<5	9.70	2.3	57.3
25	75	60.8	77.1	0.789	8.4	57.1	<5	9.77	2.4	53.3
50	50	59.8	71.2	0.840	9.5	42.2	<5	7.50	1.4	32.1

TABLE VIII

ANALYSIS OF NSSC SPENT LIQUOR — CENTRIFUGED AND FILTERED

		As-Received Basis	Ovendry Solids Basis
Copper content, %		0.000032	0.00025
Iron content, %		0.0025	0.019
Zinc content, %		0.00026	0.0020
Mercury content, µg/l		None[a]	
%		0.0000004	0.000003
Total carbohydrate, %		1.11	8.56
Total solids, %	Dupl. av.	12.96	
Ash, %	Dupl. av.	8.22	63.43
Phosphorus, %		None[b]	None[c]
Magnesium, %	Dupl. av.	0.0070	0.050
Manganese, %	Dupl. av.	0.0038	0.029

		As-Received Basis	Ovendry Solids Basis
Silver, %		None[d]	None[e]
Lead, %		None[f]	
Calcium, %	Dupl. av.	0.020	0.14
Molybedenum, %		None[g]	0.02
Total sulfur, %	Dupl. av.	0.76	5.86
Acetate, %	Dupl. av.	2.20	16.98
Sodium, %		1.5	12
Kjeldahl nitrogen, mg/l		<0.6	
Acid-soluble lignin, %	Dupl. av.	4.57	35.3
Acid-insoluble lignin, %	Dupl. av.	0.02	0.15

Note: Unless otherwise indicated, all values are single determinations.

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[a] Detection limit = 4.
[b] Detection limit = 0.04.
[c] Detection limit = 0.3.
[d] Detection limit = 0.00002.
[e] Detection limit = 0.0002.
[f] Detection limit = 0.0005.
[g] Detection limit = 0.003.

Methods: Copper, iron, and zinc — Atomic absorption spectrophotometry, acid digestion of sample.
Mercury — Flameless atomic absorption spectrophotometry, aqua regia method.
Total carbohydrate — Tappi 53(2):257-61(Feb. 1970).
Total solids — Dried at 105°C for 24 hours.
Ash — Ashed at 650°C for 1 hour.
Metals — Emission spectroscopy.
Total sulfur — Marathon Research Method.
Acetate — TAPPI Standard T 629 os-53.
Sodium — Flame photometry.
Kjeldahl nitrogen — Standard methods of the analysis of water and wastewater, 13th ed., p. 469, 1971.
Acid-insoluble lignin — TAPPI Standard T 13 m-54.

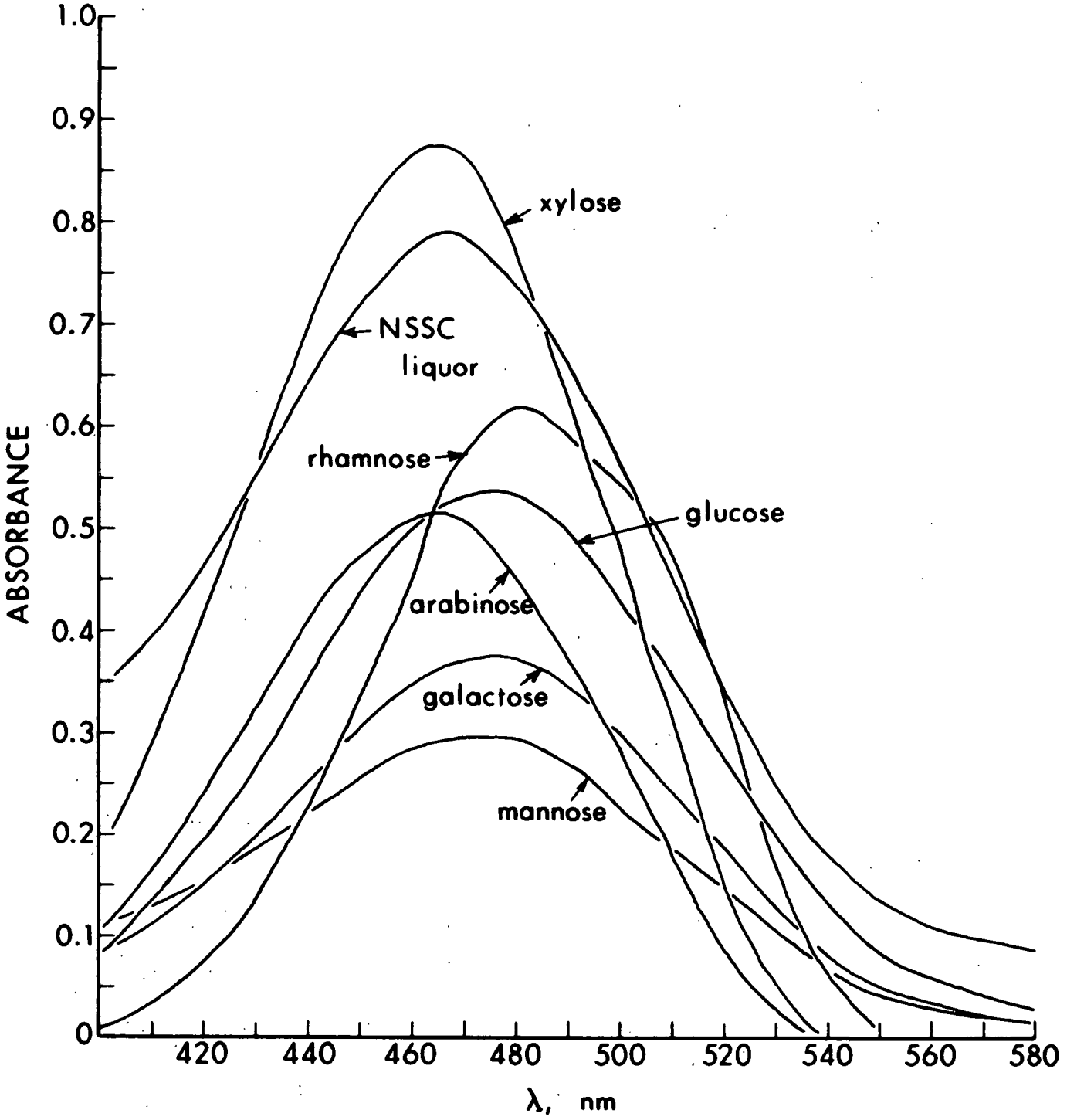

Figure 1. Visible Spectra of Colors Obtained by the Reaction of Indole with Sugar Constituents of NSSC Spent Liquor

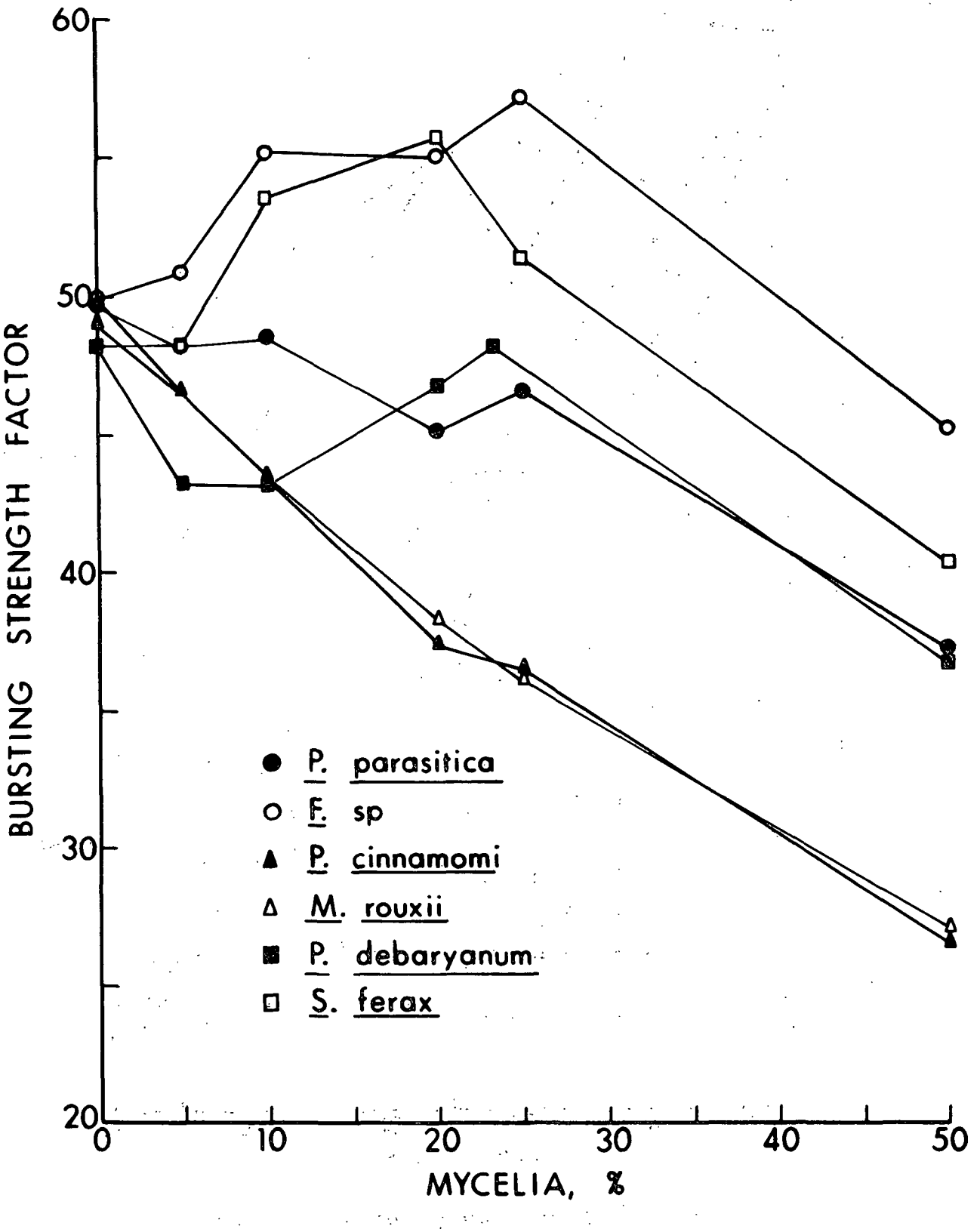

Figure 2. Bursting Strength of Mycelial Paper

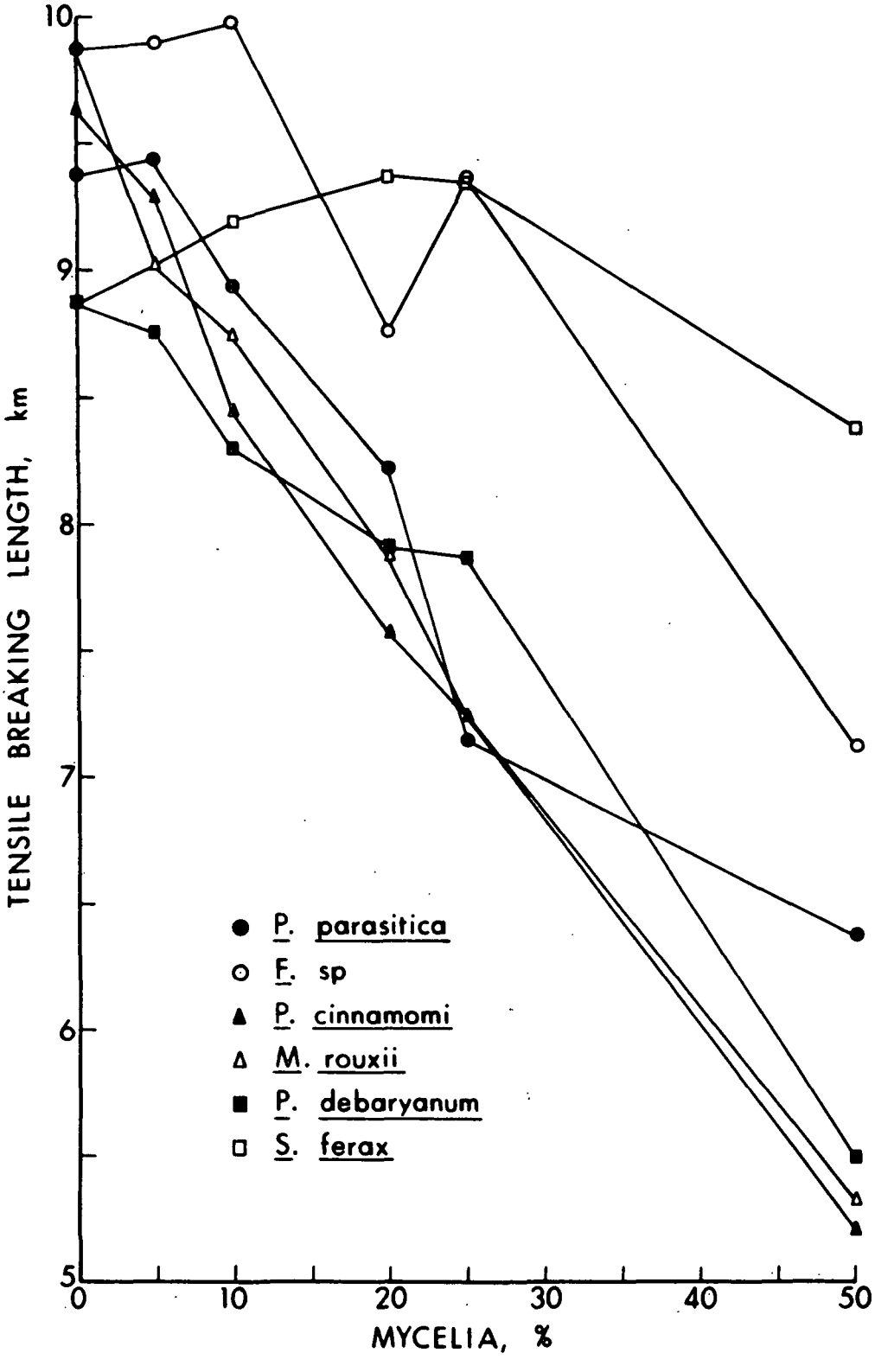

Figure 3. Tensile Strength of Mycelial Paper

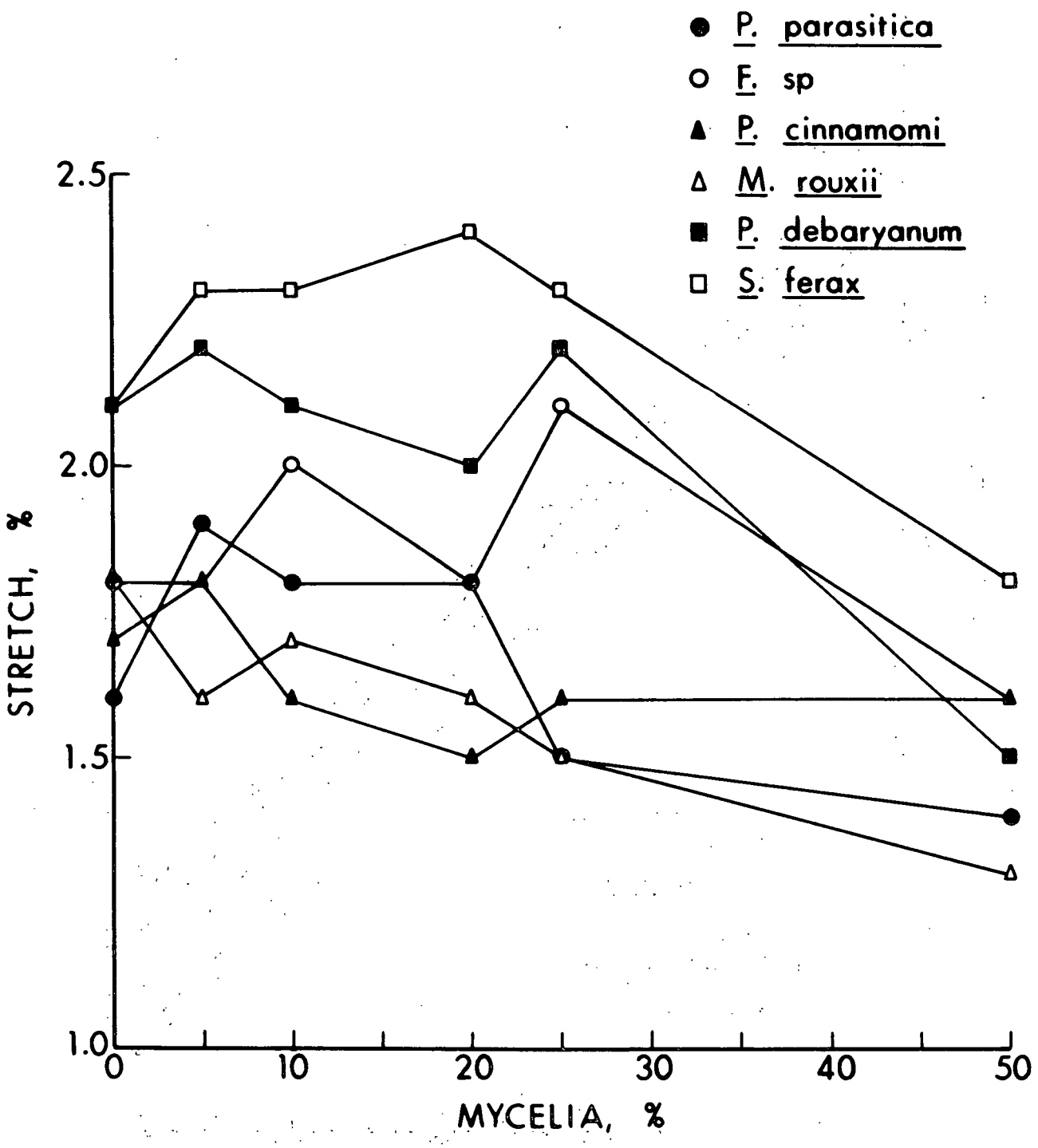

Figure 4. Stretch of Mycelial Paper

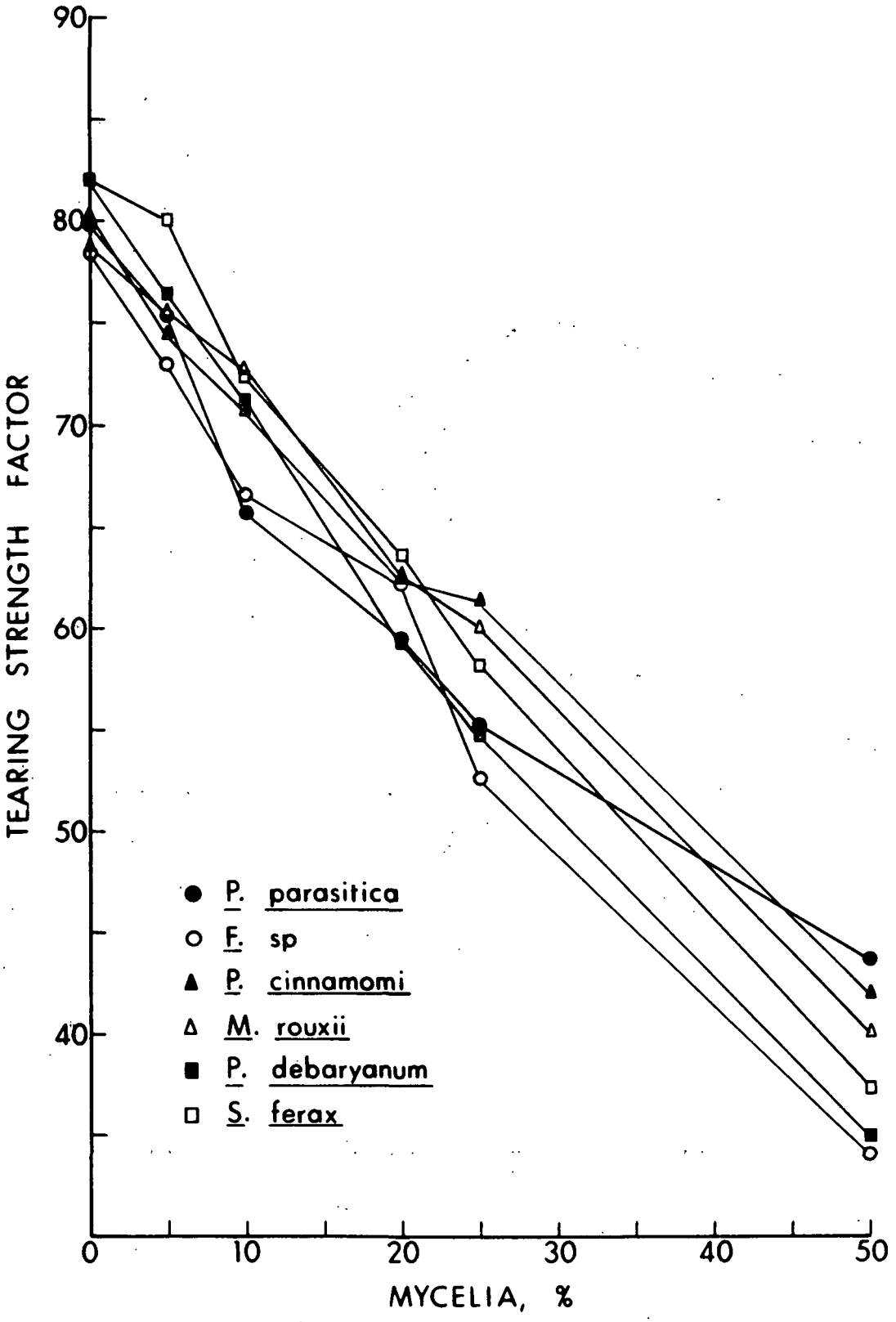

Figure 5. Tearing Strength of Mycelial Paper

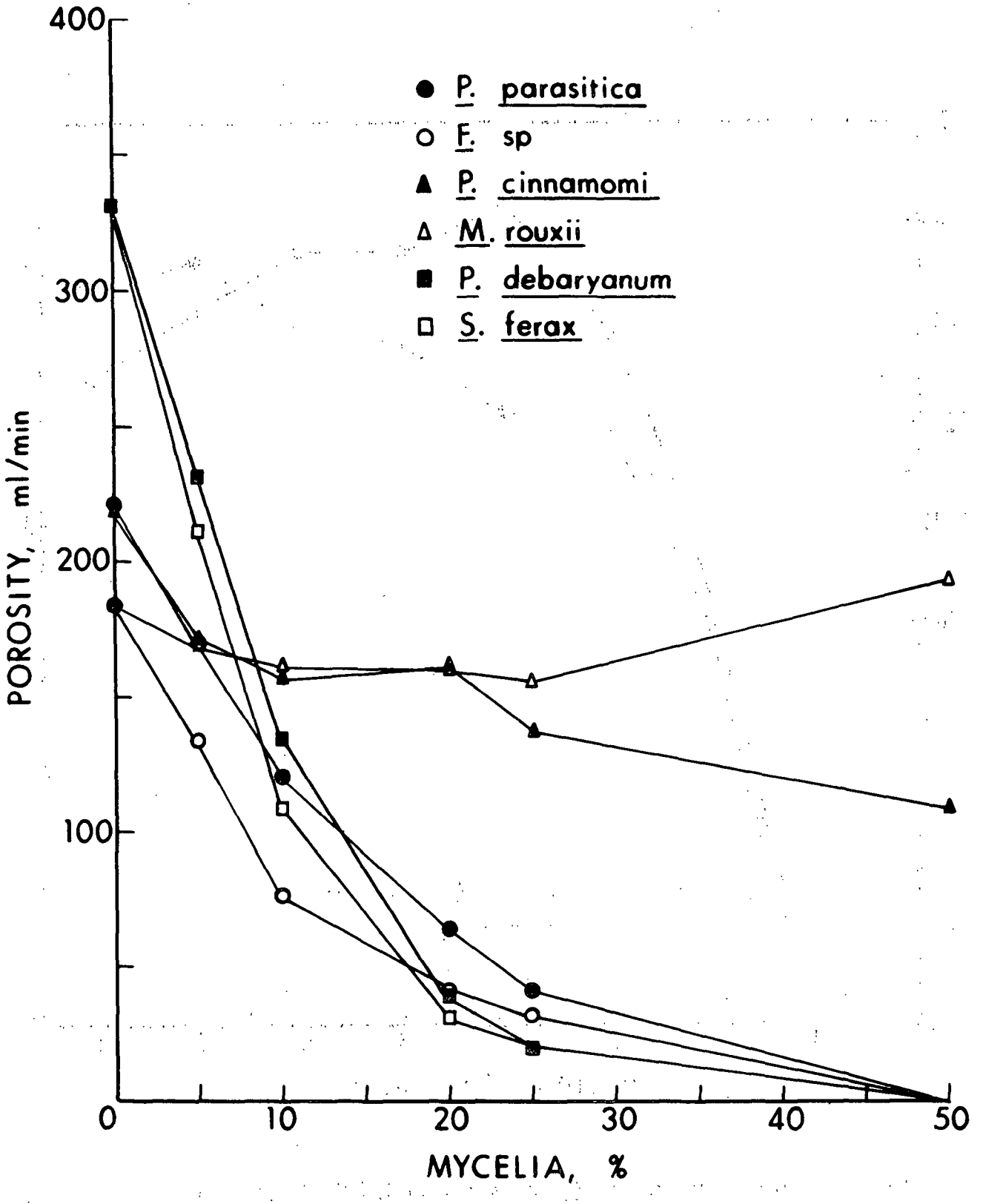

Figure 6. Porosity of Mycelial Paper

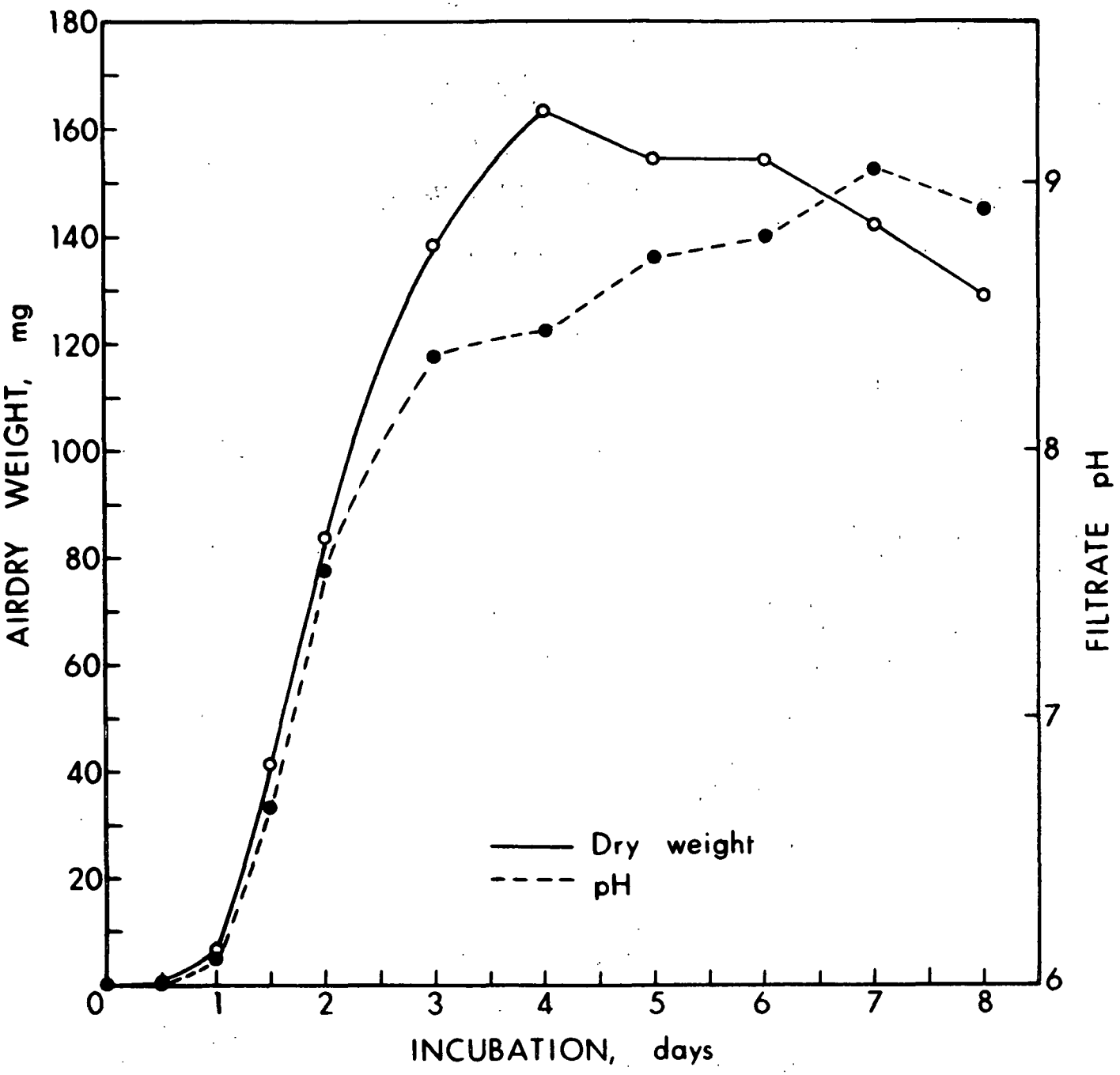

Figure 7. Growth Curve for Fusarium sp. on 25% NSSC Spent Liquor

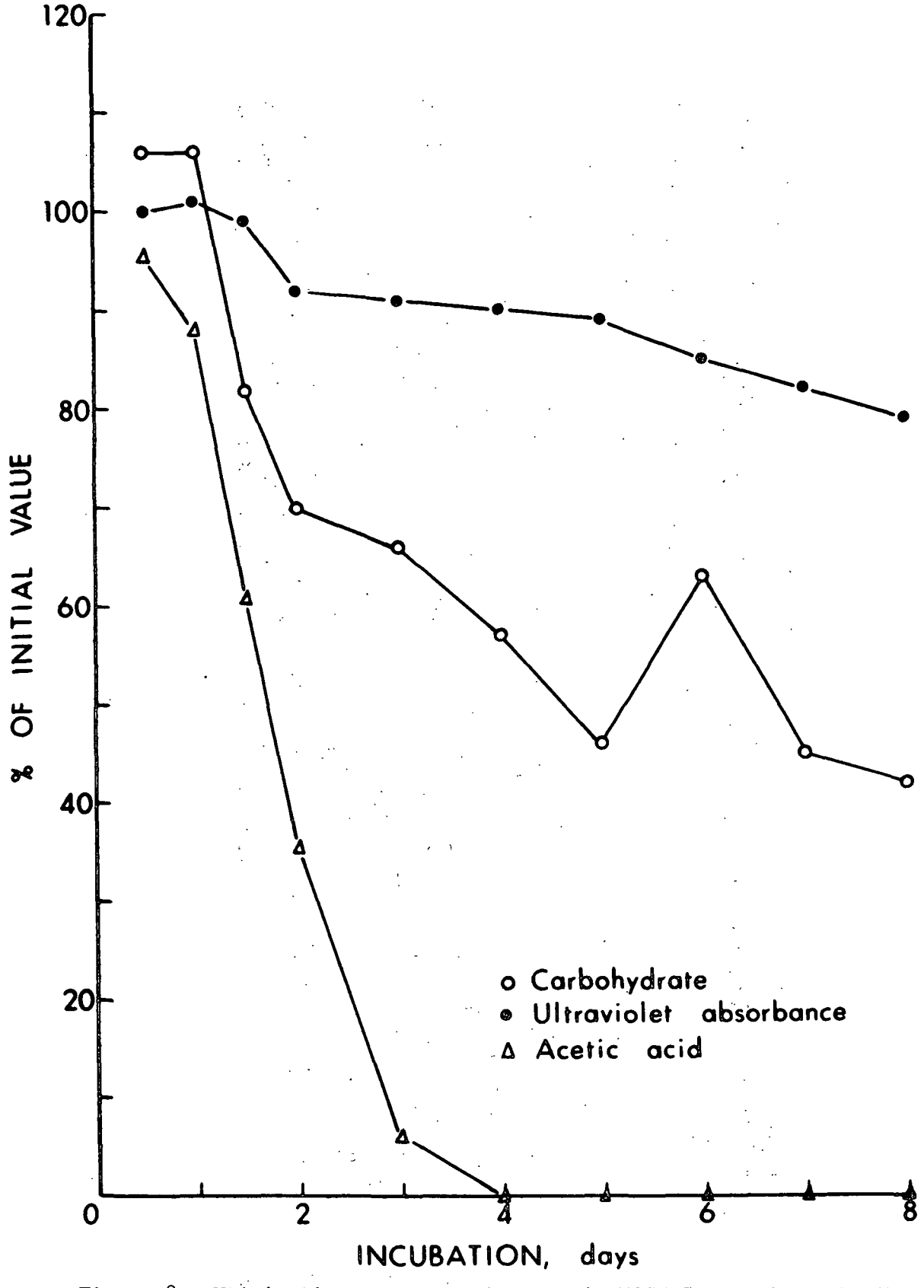

Figure 8. Utilization of Carbon Sources in NSSC Spent Liquor by Fusarium sp.

Figure 9. Scanning Electron Micrograph of an Aspen Kraft Handsheet Containing 20% P. parasitica Mycelia. Top: 300X; Bottom: 600X